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REVIEW

Microbial ecology in *Hydra*: Why viruses matter

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While largely studied because of their harmful effects on human health, there is growing appreciation that viruses are also important members of the animal holobiont. This review highlights recent findings on viruses associated with *Hydra* and related Cnidaria. These early evolutionary diverging animals not only select their bacterial communities but also select for viral communities in a species-specific manner. The majority of the viruses associating with these animals are bacteriophages. We demonstrate that the animal host and its virome have evolved into a homeostatic, symbiotic relationship and propose that viruses are an important part of the *Hydra* holobiont by controlling the species-specific microbiome. We conclude that beneficial virus-bacterial-host interactions should be considered as an integral part of animal development and evolution.

Keywords: innate immunity, host-microbe interaction, holobiont, virus, evolution, microbiota, *Hydra*

Introduction

All animals, including humans, are ecosystems comprised of a myriad of microbes including archaea, viruses, and bacteria (Qin *et al*., 2010; Human Microbiome Project Consortium, 2012; Gilbert *et al*., 2012; McFall-Ngai *et al*., 2013). The partnerships that animals have forged with microbes have been powerful agents of change. There is now a growing realization that bacteria not only enable animals to metabolise otherwise indigestible polysaccharides such as lignin and cellulose (Turnbaugh *et al*., 2006, 2008; Frankenfeld *et al*., 2014), but also shape animal development and behaviour (Stappenbeck *et al*., 2002; Fraune and Bosch, 2010; Sharon *et al*., 2010; Rosenberg and Zilber-Rosenberg, 2011; Pennisi, 2013; Hsiao *et al*., 2013; Kaiko and Stappenbeck, 2014). Interactions between the members of the holobiont, i.e. prokaryotic symbionts, eukaryotic symbionts, and host cells, have been critical to enabling the key transitions in animal evolution (Brucker and Bordenstein, 2012, 2013a, 2013b; Stilling *et al*., 2014). Today we realize that microbial communities colonize all animal epithelia; and therefore, any multicellular organism must be considered an association comprised of the macroscopic host in synergistic interdependence with the microbiome, consisting of bacteria, archaea, fungi, and numerous other microbial and eukaryotic species. We refer to these associations as "holobionts" or "metaorganisms" (Eberl, 2010; Bosch and McFall-Ngai, 2011). The metaorganism concept considers the dynamic communities of bacteria on epithelial surfaces as an integral part of the functionality of the respective organism itself (Turnbaugh *et al*., 2007; Biagi *et al*., 2011, 2012). A metaorganism can be analysed, measured and sequenced; and occupies an ecological niche, adapts, and may even be the organizational level at which natural selection acts.

The increasing appreciation that animals cannot be considered in isolation but only as a partnership of animals and symbionts has lead to two important realizations. First, it is becoming increasingly clear that to understand the evolution and biology of a given species, we cannot study the species in isolation of its symbionts. And second, the health of animals, including humans, appears to be fundamentally multi-organismal and depends on trans-kingdom interactions. Any disturbance within the complex community causes a dysbiosis and has drastic consequences for the well-being of the members.

Metaorganisms have evolved for more than 500 million years, yet only recently have advances in sequencing technology allowed us to appreciate the full nature of the complexities in host-microbe interactions. These technological advances have revealed that in addition to bacteria and associated eukaryotic symbionts, one more important player is an integral member of the holobiont: viruses. Viruses are found anywhere cellular life exists, in any environment or ecosystem (Reyes *et al*., 2012). They exist along with their hosts as part of a dynamic community ensemble of exogenous viral particles and endogenous proviruses. Yet, although being the most abundant and diverse biological component on the planet, comparatively little is known about their role in the holobiont (Reyes *et al*., 2013). Does the virome play a role in establishing and maintaining the microbiome? What are the underlying mechanisms as to how viruses control microbes? Can bacteriophage-based therapies be used in invertebrates such as corals for treating complex disorders?

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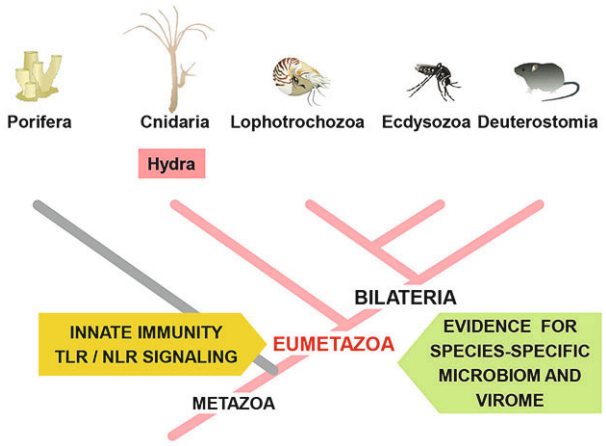

Fig. 1. Cnidarian *Hydra* are basal eumetazoans, yet maintain innate immune signaling cascades and species-specific holobiont interactions.

In this review, we will focus on insights gained mostly from the *Hydra* model that have revealed that viruses and bacteriophages are abundant and important components of the holobiont.

Rationale for studying host-microbe interactions in *Hydra*

High-throughput sequencing technologies have dramatically and fundamentally altered our ability to characterize biodiversity. Metagenomic sequencing has demonstrated that individuals from *Hydra* to human are not solitary, homogenous entities but consist of complex communities of many species that likely evolved during a half-billion years of coexistence (Human Microbiome Project Consortium, 2012; McFall-Ngai *et al.*, 2013). Understanding the relationships between the different members of a given holobiont has helped to form an integrated view of an organism. From an evolutionary and ecological perspective, the complexity of these issues at the interface with the microbial environment is invigorating. But within that complexity lie answers to the fascinating question of how trans-kingdom interactions have so profoundly shaped the evolution of life.

Given that multiple interconnected factors with complex inter-relationships between both macroscopic host and microbiota (bacteria, archaea, viruses) are at play, it is important to use a genetically tractable and morphologically simple model host. Additionally, this host should associate with a limited number of microbial species, which can be cultured independently, allowing one to deconstruct the complex host-microbe interactions in great detail. Such a model can function as a living test tube, and may be a key to dissecting the fundamental principles that underlie all host-microbe interactions. And if such a model represents an ancient animal phylum, it will reveal important insights not only into host-microbe interactions but also into the evolutionary guiding principles underlying the recognition, maintenance and colonization processes that are also relevant to a mammalian holobiont.

One such phylogenetically ancient organism that fits this criteria is the *Hydra*, a member of the phylum Cnidaria (Bosch, 2012a, 2012b, 2013, 2014). *Hydra* has been used successfully as a model host for the study of host-microbe interactions (Fraune and Bosch, 2007; Bosch, 2013, 2014). *Hydra* belongs to one of the most basal eumetazoan phylum, the Cnidaria, which are a sister taxon to all Bilateria. Living in the Ediacaran oceans, Cnidaria (jellyfish, corals, and hydroids) evolved early during the phylogenesis of multicellular animals (Metazoa) (Philippe *et al.*, 2011; Nosenko *et al.*, 2013; Knoll and Sperling, 2014; Mills *et al.*, 2014). Cnidarians not only are among the earliest known phyletic lineages to form natural symbiotic relationships with bacteria and eukaryotes (Fig. 1), but also possess most of the gene families found in bilaterians and have retained many genes that have been lost in *Drosophila melanogaster* and *Caenorabditis elegans* (Kortschak *et al.*, 2003; Miller *et al.*, 2005; Technau *et al.*, 2005; Putnam *et al.*, 2007; Hemmrich *et al.*, 2012). For this reason, these "basal metazoans" allow us to gain insights into the very early evolution of mechanisms that may control host-microbe interactions.

Hydra represents a classical model organism in developmental biology, which was introduced by Abraham Trembley in 1744. *Hydra* have a simple body plan (Fig. 2), consisting of only two epithelial layers (an endodermal and ectodermal epithelium separated by an extracellular matrix mesoglea), a single body axis with a head, gastric region and foot, and a limited number of different cell types. *Hydra* served for many years as a model in developmental biology to approach basic mechanisms underlying *de novo* pattern formation, regeneration, and cell differentiation. Molecular tools including transgenesis (Wittlieb *et al.*, 2006) as well as rich genomic and transcriptomic resources (Chapman *et al.*, 2010) make *Hydra* a valuable system, which can be manipulated experimentally. Accessibility to both gain-of-function and loss-of-function experiments allow for thorough analysis of molecular pathways. Because of its simplicity in body structure and its exclusive reliance on the epithelial innate immune system, the maintenance of epithelial barriers can be

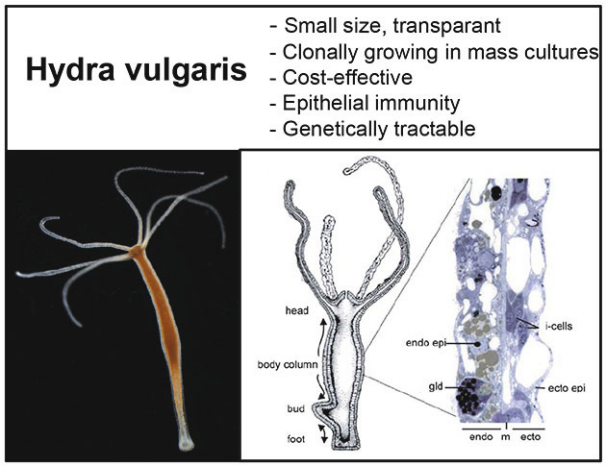

Fig. 2. Schematic view of *Hydra* indicating the simple body plan consisting of a tube-like directive axis with an oral ("head") and an aboral ("foot") end.

investigated in the absence of the adaptive immune system and other immuno-related cell types and organs. Moreover, due to the relatively simple microbial community structure associating with the animal, which consists of only few bacterial phylotypes (most of which can be cultured *in vitro*), the influence of the microbiota under healthy and disease condition can be dissected. Finally, since nearly all known genes involved in innate immunity are present in *Hydra*, the uncovered basic molecular machinery can be translated to more complex organisms including humans.

Hydra – bacteria interactions

Bacteria in *Hydra* are specific for any given species (Fraune and Bosch, 2007). Closely related *Hydra* species such as *Hydra vulgaris* and *Hydra magnipapillata* are associated with a similar microbial community. In line with this, comparison of the phylogenetic relatedness of *Hydra* species with that of cognate associated bacterial communities reveals a high degree of congruency (Franzenburg *et al*., 2013a). Since the composition of the microbiome in *Hydra* parallels the phylogenetic relationships of *Hydra* species, *Hydra*'s microbiome reflects an ancestral footprint of evolution – a pattern termed phylosymbiosis (Brucker and Bordenstein, 2013b, 2014).

Based on Roche 454 pyrosequence analysis of 16S rRNA genes, the dominant bacteria species (75% relative abundance) in the laboratory strain *Hydra vulgaris* (AEP) is *Curvibacter* sp. (Fraune and Bosch, 2007; Fraune *et al*., 2014). *Hydra vulgaris* AEP has about 150 operational taxonomic units (OTUs) per polyp according to the chao1 index (based on 97% similarity), indicating that beside the dominant *Curvibacter* population, bacterial diversity within the remaining 25% of bacteria is still quite high. Members of the microbiome include *Duganella* sp. with an abundance of 11%; *Undibacterium* sp. with a relative abundance of 2%; *Acidovorax* sp. with an abundance of 0.7%; *Pelomonas* sp. (abundance of 0.2%); and *Pseudomonas* sp. with an abundance of 0.4% (Fraune *et al*., 2014). Since *Hydra* individuals living in the wild are colonized by a similar group of microbes to polyps grown in the lab, these animals appear to maintain specific microbial communities over long periods of time (Fraune and Bosch, 2007). Further, the stable population of *Curvibacter* sp. may be necessary for dependent metabolic conditions for the animal, while the diversity of the smaller population of bacterial species may play niche roles in metabolism according to environmental changes (Bosch, 2013).

By profiling the assembly of the microbiota on *Hydra* epithelium up to 15 weeks post-hatching, distinct and reproducible stages of colonization can be observed. High initial variability and the presence of numerous different bacterial species are followed by the transient preponderance of the bacterial species that later dominate the adult microbiota (Franzenburg *et al*., 2013b). At the end of the colonization process there is a drastic decrease of diversity.

Taken together, these findings strongly indicate that distinct selective pressures are imposed on and within the *Hydra* epithelium. This suggests that each *Hydra* species actively shapes their specific bacterial community and that *Hydra*´s microbiota is a complex trait that is under strong host genetic control.

Beneficial viruses

Metagenomic approaches have transformed our understanding of viruses in many ecosystems, significantly advancing our knowledge of diversity, abundance, and virus-host interactions. Outside of pathogenic effects, little is still known about viruses infecting eukaryotic hosts. In spite of the common perception of viruses as pathogens, many viruses are in fact beneficial to their hosts in various ways. Beneficial viruses have been discovered in many different hosts, including bacteria, insects, plants, fungi and animals (Roossinck, 2011). For example, many parasitoid wasps species are known to harbor symbiotic viruses, and these viruses have a mutual relationship with their wasp host, particularly for host immune responses (Renault *et al*., 2005). As comprehensively reviewed by Roossinck (2011), the beneficial effects of viruses range from obligate mutualisms, in which the survival of the host is dependent on the virus, to benefits that occur only under specific environmental conditions. In addition, some of these relationships are ancient and the line between the virus and its host is blurry, and as the relationship between the aforementioned wasps and polydnaviruses has shown, some relationships are clearly symbiogenic. It seems that beneficial viruses have played a major part in the evolution of life on earth. However, how these beneficial interactions evolve is still a mystery in many cases.

A symbiotic function for viruses has only recently been demonstrated in mammals. Norovirus has been shown to compensate for the presence of the microbiome in germ-free mice (Kernbauer *et al*., 2014). A functional immune system was necessary for this compensation indicating that viral-host interactions are vital for animal health. In another study, Hoc-domain containing T4 bacteriophages have been shown in a microarray study to have the potential to interact with glycan residues decorating mucin glycoproteins using capsid proteins containing immunoglobulin-like (Ig-like) folds (Barr *et al*., 2013). This provides a mechanism for viral binding to mucus leading to mucin activation of the host immune system and antimicrobial peptide secretion (Liévin-Le Moal and Servin, 2006). Furthermore, the presence of bacteriophages in the mucus, in addition to host secreted antimicrobial peptides, may allow for selection of the associated bacteria in the holobiont.

A recent study reveals that viral communities in *Hydra* are species-specific

Using a metagenomic approach we have analyzed five different *Hydra* species, under non-stressed and heat-stressed conditions, for a total of 10 viromes (Grasis *et al*., 2014). Taxonomic evaluation of the viral families isolated from lab-cultured or wild-caught *Hydra* revealed that each species of *Hydra* are associated with a diverse community of prokaryotic and eukaryotic viruses. The cladogram shown in Fig. 3 shows very distinct viral populations associating with each

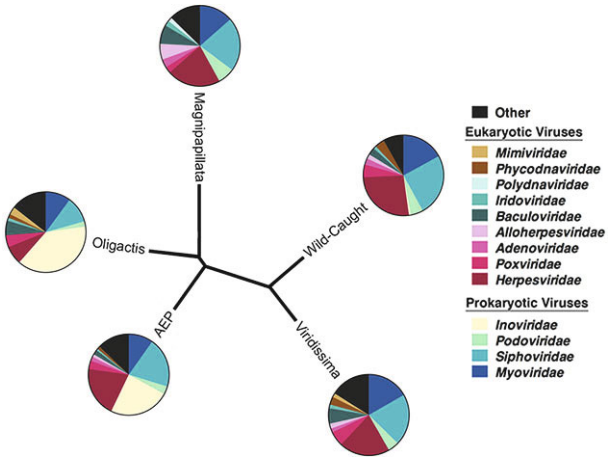

Fig. 3. ***Hydra*** **phylosymbiotic associations with viral families.**

of the *Hydra* species, suggesting a phylosymbiotic pattern (Grasis *et al.*, 2014). The most common viral families associating with *Hydra* are prokaryotic viruses belonging to the *Myoviridae*, *Siphoviridae*, and *Inoviridae* families, as well as the eukaryotic viruses belonging to the *Herpesviridae* family. These four families of viruses accounted for 43–74% of the viral sequences found to be associated with various *Hydra* species. The *Herpesviridae* family accounts for 18–53% of the total population of associating eukaryotic viruses in *Hydra* species. Outside of these families, a wide diversity of viruses unique to each species of *Hydra* was observed.

The largest percentages of associating viruses for each species of *Hydra* were found to be prokaryotic viruses, which infect the bacterial and archaeal domains. Predicted prokaryotic hosts accounted for 38–63% of the viral sequencing hits. The two most common families of bacteriophages associating with most *Hydra* species are the *Myoviridae* and *Siphoviridae* families, accounting for 15–45% and 15–55% of the total number of bacteriophages, respectively. These results are similar to the viral communities found in the human gut, with large percentages of *Myoviridae* and *Siphoviridae* families are associated with human individuals (Minot *et al.*, 2011). In contrast, very low percentages of single-stranded DNA *Microviridae* family viruses were found in *Hydra* species, compared to large percentages found in the human gut (Minot *et al.*, 2011) and in fresh water environments (Roux *et al.*, 2012). These results suggest selection of specific viral communities from the environment by the host *Hydra* species useful for its regulation of the microbiome. Further, this selection indicates that the viruses associating with the host *Hydra* are not merely a reflection of the viruses present in the local water environment.

Hydra-associated viruses are not only species-specific but also sensitive to temperature stress. In all investigated *Hydra* species, heat-stress increased the diversity of viral families associated with the animal, according to a Shannon-Wiener index analysis (Grasis *et al.*, 2014). Interestingly, in each species, heat-stress also caused the diversity of bacteriophage hosts to increase. The bacteriophage viromes, however, remained relatively unchanged upon heat-stress, with only the *H. vulgaris* (AEP) strain displaying a reduction in its primary associating bacteriophage family of *Inoviridae* to two distinct populations of *Myoviridae* and *Siphoviridae*. Taken together, environmental stress causes a shift in the community of viruses associated with each *Hydra* species, particularly amongst the eukaryotic viral population. We conclude that the animals dynamically regulate their associated viral communities to compensate for changing environmental factors.

This observation of temperature stress leading to increased host-associated viral diversity points to the presence of a diverse latent viral community, which can be reactivated by environmental stress and start the lytic part of the viral life-cycle. Environmental induced changes in the life cycle of viruses characterised by a dormant state and active proliferation of viral particles is known from different human pathogenic viruses belonging to *Herpes-* and *Poxviridae*, both also found in the viromes of *Hydra*. Establishment and maintenance of latency as well as stress-mediated viral replication are complex interactions of protein-protein interactions and post-transcriptional regulation of viral gene expression (Umbach *et al.*, 2008; Li *et al.*, 2011). Depending on the outcome of this battle, viral progeny infect new cells or individuals, and regain homeostasis by latency or establish a chronic infection with a continuous proliferation of viral particles. These viral host interactions can be negative (pathogenic), but can also be beneficial to the host. Chronic viral infections with a continuous proliferation of viral particles have been

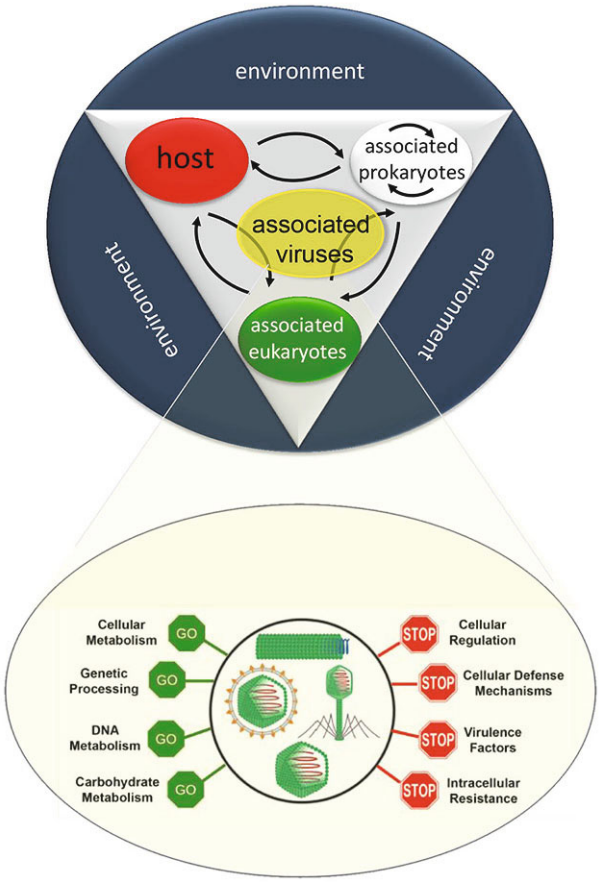

Fig. 4. **Schematic view of the interactions between host organisms, their microbiota and viruses.**

shown to protect their host from secondary viral or bacterial infections (Barton *et al*., 2007; Virgin *et al*., 2009). Chronic infection induced protection is unspecific and based on the up-regulation of the basal state of innate immunity (Duerkop and Hooper, 2013). How viruses interact with the innate immune system of *Hydra* and affect the immune response towards its microbiota remains to be uncovered.

Functional metabolic community profiles of the viromes associating with each species of *Hydra* provided evidence that many cellular functions in the holobiont are affected by the increase in viral diversity upon heat-stress (Grasis *et al*., 2014). Generally, cellular metabolism and cellular genetic processing subsystems were increased, while cellular defense mechanisms were compromised (Fig. 4). Specifically, cellular DNA and carbohydrate metabolism subsystems were increased with heat-stress, while cellular virulence and defense mechanisms were nearly ablated upon heat-stress. The increase of cellular metabolism and cellular genetic processing, as well as compromised cellular defense mechanisms, indicates viral involvement in the regulation of the host-associated microbiota under these conditions.

Aquatic environmental organisms like *Hydra* are permanently exposed to diverse bacteria and viruses from the surrounding plankton community. In contrast to terrestrial organisms, transmission of potential pathogens is independent from vector organisms and imposes an increased risk of infection and of viral host switching. Viral host switching is commonly observed in bacteriophages infecting individual hosts from different species and even genera (Weitz *et al*., 2013). In bacterial communities, bacteriophage induced cell lysis, also termed 'phage predation', has a tremendous impact on bacteria affecting 20–40% of mortality (Suttle, 2007). However, even under these conditions, *Hydra* is able to preserve its specific bacterial and viral communities. Beside the innate immune system of *Hydra*, which is essential to establish and maintain a host-specific bacterial community (Bosch, 2014), host-specific bacteria feature its own defence mechanisms to protect themselves from bacteriophage infection. Some *Hydra*-associated bacteria exhibit both CRISPR (clustered regularly interspaced short palindromic repeat) adaptive immunity and restriction-modification (RM) system to degrade foreign DNA. Moreover, genome sequencing of *Hydra*-associated bacteria revealed the presence of prophages within several bacterial genomes (S. Fraune, personal communication). If the presence of prophages prevents other bacteriophages from infecting and thus excludes superinfection (Stern and Sorek, 2012) and/or if prophages are internal regulators to control the abundance of *Hydra*-associated bacteria is so far unclear.

We conclude that environmental stress dramatically changes the interaction between host and viruses and that *Hydra* dynamically regulates this interaction and selectively shapes its virome to regain homeostasis. This suggests that genetic factors of the host can outweigh high mutation rates of viruses and select for 'beneficial' viral variants. Taken together, viral replication and host selection of both *Hydra* and associated bacteria add to the adaptation of the holobiont towards changing environmental conditions (Fig. 4). The forces that shape the virome composition and beneficial effects of horizontal gene transfer remain to be uncovered.

Implications for understanding the *Hydra* holobiont

Up to now, viruses were mostly considered as pathogenic agents. However, ideas about the role of viruses in multicellular organisms are changing, and might be undergoing a paradigm shift. Our findings that multicellular animal hosts such as *Hydra* are associated with a unique viral community helps to provide a new basis for understanding the role of viruses in animal evolution and extends the concept of phylosymbiosis to include viral involvement (Brucker and Bordenstein, 2012, 2013b; Grasis *et al*., 2014). Many selective forces may influence the composition of the virome within the holobiont, including microbe-microbe interaction(s), host-derived factors, nutrients, and pressures from the external environment. An intriguing future question is the nature of the forces that shape the specific viromes. Using the *Hydra* system and its experimental tractability will further our understanding of the impact of the virome on host physiology and stability of the holobiont.

Bacteriophage therapy in corals?

The use of bacteriophages for the treatment of human disease has had a long and rocky history (Lederberg, 1996; Sulakveldize *et al*., 2001; Chanishvili, 2012; Verbecken *et al*., 2014). The search for alternatives to antibiotics has led many scientists to a treatment practice that has been on the fringes of modern medicine for nearly a century. Bacteriophages were first used in 1919 to treat a wide range of infections. Classically, the treatment uses a bacteriophage, or cocktail of several bacteriophages, to specifically lyse target pathogenic bacteria (Chanishvili, 2012). In the context of normally associating bacterial and viral communities associating with the host, the presence of pathogenic bacteria causing disease-like states represents a dysbiosis of these normally associating communities. Bacteriophage therapy, therefore, aims to restore the normal symbiosis through specific lysis of the pathogenic bacteria, leading to increased homeostasis.

Viruses and bacteriophages are also a common component of the Cnidarian coral reef environment (Rosenberg *et al*., 2007). Intriguing recent studies indicate that coral diseases such as Coral White Plague Disease may be managed by bacteriophages to reduce bacterial infection (Efrony *et al*., 2008). The rapidly progressing white plague-like disease of the coral *Favia favius* is caused by the gram-negative pathogen *Thalosomonas loyaeana* (Barash *et al*., 2005; Thompson *et al*., 2006). In the control group, with no bacteriophage addition, the coral tissue infected with *T. loyaeana* was observed to be lysed after only 4–6 days. When corals were treated with bacteriophage BA3 1 day after *T. loyaeana* infection, they were protected from further infection for at least 37 days. However, corals inoculated with bacteriophage BA3 2 days after infection with *T. loyaeana* were not protected, indicating that bacteriophage therapy may be able to prevent the spread of the disease, rather than cure an already infected coral.

A related study investigated the feasibility of applying bacteriophage therapy to treat the coral pathogen *Vibrio cor-*

alliilyticus (Cohen *et al.*, 2013). A specific bacteriophage for *V. coralliilyticus* strain P1, a lytic bacteriophage belonging to the *Myoviridae* family and termed bacteriophage YC, was isolated from the seawater above corals. Bacteriophage therapy experiments using coral juveniles in microtiter plates as a model system revealed that bacteriophage YC was able to prevent *V. coralliilyticus*-induced photo-inactivation and tissue lysis.

Whether bacteriophage therapy becomes a mainstream approach to address the global problem of coral diseases remains to be shown. Major advantages of bacteriophage therapy of coral diseases include host specificity, self-replication and environmental safety. The bacteriophage only attacks and destroys the specific pathogen, leaving the remaining beneficial microorganisms untouched. However, due to mutation rates of viruses, scientists stress that it is uncertain how bacteriophage therapy will progress in the field (Ben-Haim and Rosenberg, 2002; Ben-Haim *et al.*, 2003a, 2003b; Cohen *et al.*, 2013). They suggest "clinical field trials" to determine the efficacy of bacteriophage therapy in treatment of Coral White Plague disease. On the other hand, it is probably most important to understand the factors contributing to disease development and progression. In this context environmental stress has a fundamental effect on the stability and composition of the holobiont, making it more susceptible to pathogen infections. Stress-induced changes, and the resulting imbalance of the holobiont, are important factors in elucidating complex diseases and not only in cnidarians.

Bacteriophage therapy in human medicine fell out of favor with the advent of antibiotics; the practice has only persisted in some European countries as an experimental treatment (Chanishvili, 2012). However, recently bacteriophage therapy was highlighted as one of seven approaches to "achieving a coordinated and nimble approach to addressing antibacterial resistance threats" in a 2014 status report from the National Institute of Allergy and Infectious Diseases (NIAID, USA). Renewed interest in bacteriophage therapy is due in part to the growing problems posed by antibiotic overuse in the clinic, which has escalated microbial resistance (Verbeken *et al.*, 2014). With antibiotic resistant microbes on the rise, it may now be the time to reconsider bacteriophage therapy as a viable addition to antibiotics.

Conclusions

There remain many unanswered questions in the role and use of viruses in animal hosts, and many of these questions are especially tractable using simple invertebrate model systems such as *Hydra*, which allow for the molecular dissection on both the host and the microbial fronts. By virtue of its morphological simplicity and molecular accessibility, *Hydra* allows thorough *in vivo* analysis of virus-bacteria-host interactions. Host factors required for virus colonization can be identified. The use of bacterial genetic tools and the isolation of *Hydra* bacteriophages from distinct bacterial hosts will help elucidate the specific microbe-derived factors. Taken together, *Hydra* offers a fascinating model to help understand that an organism's genetics and fitness are inclusive of its virome and microbiome.

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